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PARACETAMOL METABOLISM IN ALCOHOLICS AND PATIENTS
ON ANTICONVULSANT OR ANTITUBERCULOUS THERAPY

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SUMMARY

1. The metabolism of paracetamol after a single oral dose of 20 mg/Kg was compared in fifteen patients with microsomal enzyme induction taking anticonvulsants or rifampicin, sixteen alcoholics defined by their M.A.S.T. scores and twelve healthy volunteers.

2. The plasma antipyrine half life was used to assess induction and the means were 6.4, 13.4, 12.8 hours in the three groups respectively

3. As well as a reduced antipyrine half life, the induced patients showed enhanced glucuronide conjugation of paracetamol with correspondingly reduced excretion of the sulphate conjugate and unchanged drug.

4. The alcoholics did not have significantly different antipyrine half lives or indices of glucuronide conjugation compared to the healthy volunteers.

5. There were no significant differences between the means of the three groups in the urinary excretion of the mercapturic acid and cysteine conjugates of paracetamol. However, four of the alcoholics had unusually high outputs of these two conjugates.

6. Conversion of paracetamol to its potentially hepatotoxic metabolite does not appear to be increased in patients induced with anticonvulsants or rifampicin. Whether this also applies to alcoholic patients remains unresolved and further work is planned.

INTRODUCTION

Paracetamol is a widely used and normally very safe analgesic. However, in overdosage it can cause acute hepatic necrosis through the formation of a highly reactive intermediate metabolite by hepatic cytochrome P-450 dependent microsomal enzymes (Mitchell, Jollow, Potter, Davis, Gillette & Brodie, 1973; Mitchell, Thorgeirsson, Potter, Jollow & Keiser, 1974). In most laboratory animal species, the hepatotoxicity of paracetamol is increased by pretreatment with microsomal enzyme inducers such as phenobarbitone, 3-methylcholanthrene and ethanol and decreased by inhibitors such as piperonyl butoxide (Mitchell et al., 1973; Mitchell et al., 1974; Jollow et al., 1974; Strubelt, Obermeier & Siegers, 1978; Teschke, Stutz & Strohmeyer, 1979; Streeter & Timbrell, 1979). There have been recent case reports of liver damage following the alleged therapeutic use of paracetamol in chronic alcoholics (Licht, Seeff & Zimmerman, 1980; Gerber, Kaufmann, Klion & Alpert, 1980; Goldfinger, Ahmed, Pitchumoni & Weseley, 1978; McClain, Kromhout, Peterson & Holtzman, 1980). After overdosage the severity of liver damage appears to be greater in chronic alcoholics and patients who have previously been taking drugs likely to cause induction (Wright & Prescott, 1973).

These observations suggest that microsomal enzyme induction might increase the production of the reactive metabolite of paracetamol and thus enhance its hepatotoxicity. A detailed comparison of paracetamol metabolism was/

was therefore carried out in healthy volunteers, induced patients taking anticonvulsants or rifampicin and otherwise healthy males with a history of regular substantial alcohol ingestion. Microsomal enzyme induction was assessed by measurement of the antipyrine half-life. The urinary excretion of mercapturic acid and cysteine conjugates of paracetamol was used as an index of the extent of its conversion to the potentially toxic metabolite.

METHODS

SUBJECTS

Induced patients

Of the fifteen patients taking hepatic microsomal enzyme inducing drugs, thirteen were epileptics on long term therapy with anticonvulsants given singly or in combination - twelve were taking diphenylhydantoin (mean daily dose 315 mg), two phenobarbitone, two carbamazepine and one primidone. Another two patients were taking rifampicin (600 mg) daily with isoniazid for tuberculosis. All but three of the induced patients were male and their mean age and body weight were 41 years (range 22 - 72) and 71 kg (range 53 - 99) respectively.

The plasma bilirubin, alanine aminotransferase, albumin and creatinine were normal in all the patients but four had mild elevation of the alkaline phosphatase.

Alcoholic patients

Sixteen males were recruited by advertisement and by personal contact. All gave a history of regular daily consumption of at least half a bottle of spirits or six pints of beer. None was taking any other drugs regularly. Their mean age and body weight were 40 years (range 25 - 64) and 74 kg (range 59 - 95) respectively. A drug, alcohol and medical history was taken and as an independent index of alcohol consumption a Michigan Alcoholism Screening Test (M.A.S.T.) questionnaire was completed (Favazza and Pires, 1974). In all sixteen subjects this revealed at least clear evidence of alcohol related problems. Four scored into the substantial and one into the severe evidence groups. Six subjects had raised plasma gamma glutamyl transferase enzyme concentrations but only in two were they above 100 u/l (116 & 112 u/l).

Healthy volunteers

None of the twelve healthy volunteers took drugs or excessive amounts of alcohol regularly. Eleven were male and their mean age and body weight were 31 years (range 21 - 45) and 67 kg (range 55 - 82) respectively.

EXPERIMENTAL DETAILS

Drug administration and sampling regimes

The induced patients and alcoholic patients were admitted to hospital for the two day study.

On the first day, after an overnight fast, 20 mg/kg

20 mg/kg of paracetamol dissolved in 400 ml of Coca Cola was ingested over two minutes. 10 ml venous blood samples were taken over the next eight hours at $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4, 6, and 8 hours and all urine collected for the next twenty four hours with the following collection periods - 0 - 4, 4 - 8, 8 - 12 and 12 - 24 hours.

On the second day, after an overnight fast, 18 mg/kg of antipyrine dissolved in 400 ml of orange juice was ingested over two minutes. Venous blood samples (10 ml) were taken over the next twenty four hours at 0, 4, 8, 12 and 24 hours but no urine was collected.

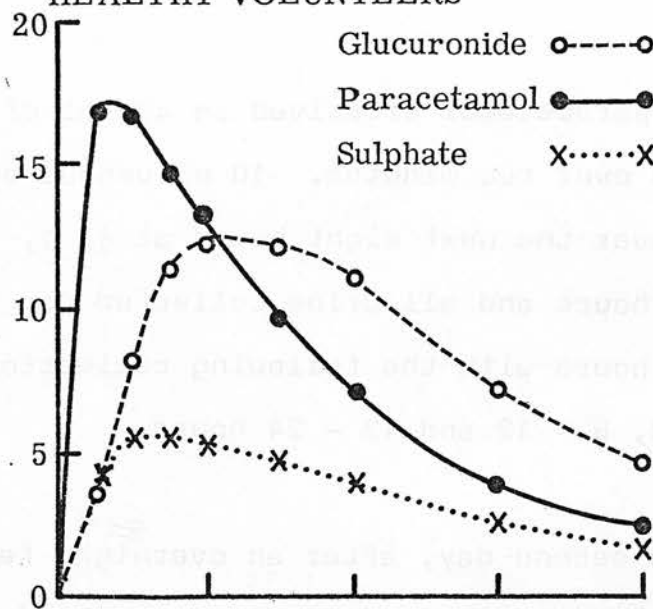
The Coca Cola and orange juice were used to disguise the taste of the drugs. All the subjects were ambulant. Fluids and tobacco were withheld for two hours and food for four hours after dosing.

The induced patients continued to receive their medications during the study. To avoid problems with alcohol withdrawal, the alcoholic patients were given 100 ml whisky at approximately 5 pm and 10 pm on both days.

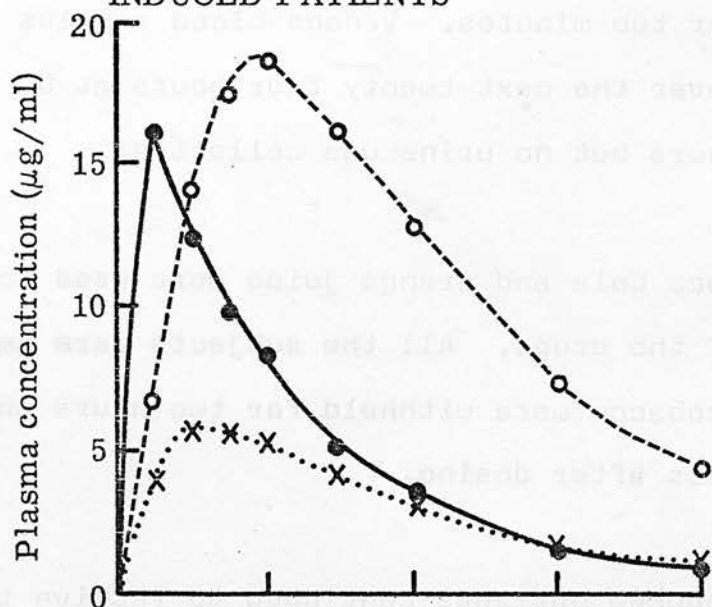
Drug analysis

Plasma and urine were stored frozen. Paracetamol and its metabolites in plasma and urine and antipyrine in plasma were estimated by high performance liquid chromatography (Adriaenssens & Prescott, 1978; Prescott, King, Brown, Balali & Adriaenssens, 1979). None of the inducing drugs or their metabolites interfered with the assays.

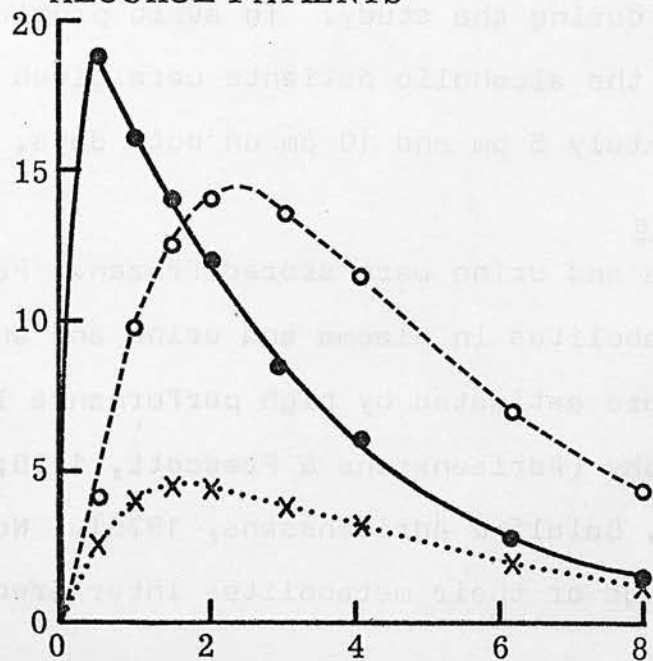
HEALTHY VOLUNTEERS



INDUCED PATIENTS



ALCOHOL PATIENTS



Hours

Calculations

The area under the plasma concentration time curves (AUC) was calculated by the trapezoidal rule, the plasma half-life from the regression of the linear terminal elimination phase and the renal clearances by dividing the amount of drug or metabolites excreted in the urine by the corresponding AUC. The apparent volume of distribution of antipyrine was obtained by dividing the administered dose by the extrapolated plasma concentration at zero time.

The plasma clearance of antipyrine was estimated by multiplying the volume of distribution by $\ln 2$ and dividing by the half-life. Paracetamol clearances were not calculated because its significant and variable first-pass metabolism would invalidate comparisons between the groups (Perucca & Richens, 1979).

Student's *t* test was used for statistical comparisons of mean data. All results are given as means \pm s.d. and concentrations of paracetamol metabolites are expressed as paracetamol equivalents.

RESULTS

Antipyrine half-life

Induction of microsomal enzymes in the patients on anticonvulsants or rifampicin was confirmed by their mean antipyrine plasma half life of 6.4 ± 2.1 h compared to that of 12.8 ± 3.9 h in the healthy volunteers. ($P < 0.001$). However there was no significant difference between the half life of the latter group and that of the alcoholic

TABLE 1. Plasma paracetamol half-life and areas under the plasma concentration time curves (AUC)
from 0 - 8 h for paracetamol and its glucuronide and sulphate conjugates

	Plasma paracetamol half life (h)	AUC paracetamol (ug/ml/h)	AUC paracetamol glucuronide (ug/ml/h)	Ratio of AUC glucuronide/ paracetamol	AUC paracetamol sulphate (ug/ml/h)
Healthy volunteers (n = 12)	2.4 ± 0.3	65 ± 9	69 ± 24	1.1 ± 0.5	28 ± 7
Alcohol patients (n = 16)	2.0 ± 0.3	58 ± 10	72 ± 23	1.3 ± 0.4	22 ± 7
Induced patients (n = 15)	1.9 ± 0.2*	40 ± 9*	88 ± 28	2.3 ± 0.7*	25 ± 8

* Significantly different from normal volunteers (P = <0.001)

alcoholic patients. (13.4 ± 5.0 h).

The mean apparent volumes of distribution in the healthy volunteers, induced patients and alcoholic patients were very similar and were 0.66, 0.65 and 0.69 l/kg respectively.

Paracetamol and Metabolites in Plasma

The mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates in the three groups are shown in the Figure.

In the induced patients paracetamol metabolism was enhanced compared to the control volunteers. The former had lower mean plasma paracetamol concentrations at all time points, a significantly smaller AUC 0 - 8 h and the plasma half life was significantly shorter (Table 1). These differences were due to increased glucuronide conjugation of paracetamol in the induced patients since the two groups had similar AUC 0 - 8 h's for the sulphate conjugate but the initial glucuronide concentrations and AUC 0 - 8 h was higher in the induced patients. The ratio of the AUC 0 - 8 h's of paracetamol glucuronide to the parent drug in the induced patients was more than double that in the healthy volunteers (Table 1).

There were no significant differences between the alcoholic patients and the healthy volunteers in any of the plasma indices of paracetamol metabolism (Table 1)

TABLE 2. Urinary excretion of paracetamol and its glucuronide, sulphate, mercapturic acid and cysteine conjugates

	Percentage excreted in 24 h as:-				% of dose recovered in 24 h
	Paracetamol	Glucuronide	Sulphate	Mercapturic acid	Cysteine
Healthy volunteers (n = 12)	4.8 ± 1.6	57 ± 11	30 ± 10	4.5 ± 1.2	3.7 ± 1.1
Alcohol patients (n = 16)	3.6 ± 2.1	57 ± 7	28 ± 6	6.1 ± 3.1	4.5 ± 2.8
Induced patients (n = 15)	2.4 ± 0.9***	68 ± 6**	22 ± 4*	4.2 ± 2.1	3.7 ± 1.3
					92 ± 10
					93 ± 7
					83 ± 19

Significantly different from healthy volunteers:- *p = <0.05, **p = <0.01, ***p = <0.001.

and thus no evidence of significant enhancement of the latter.

Urinary Excretion of Paracetamol and Metabolites

In the healthy volunteers about 5% of the total amount recovered in 24 hours was excreted unchanged with 57% as glucuronide, 30% as sulphate, 4.5% as mercapturic acid and 3.7% as cysteine conjugates.

In the induced patients, significantly less paracetamol was recovered unchanged and as sulphate while significantly more was excreted as glucuronide. The excretion of the mercapturic acid and cysteine conjugates was no greater in the induced patients than in the healthy volunteers (Table 2). There was no evidence of initial more rapid production of mercapturic acid and cysteine conjugates in the patients. The pattern of urinary excretion of paracetamol from 0 - 4 h and 0 - 24 h was similar and if anything the fraction excreted as mercapturic acid and cysteine conjugates from 0 - 4 h was less in the patients (3.6 & 2.5%) than in the healthy volunteers (3.8 & 2.8%).

The urinary excretion pattern in the alcoholic patients was not so clear cut. With regard to the mean percentages of paracetamol and its metabolites excreted over 24 hours, there were no significant differences between the alcoholic patients and the healthy volunteers. (Table 2). In fact the percentages of the major conjugates

TABLE 3. Renal clearances (0 - 8 h) of paracetamol and its glucuronide and

sulphate conjugates

	Renal Clearance (ml/min)		
	Paracetamol	Glucuronide	Sulphate
Healthy volunteers (n = 12)	11.9 \pm 4.9	131 \pm 22	166 \pm 29
Alcohol patients (n = 16)	9.0 \pm 4.9	146 \pm 33	228 \pm 47
Induced patients (n = 15)	9.0 \pm 5.5	116 \pm 30	138 \pm 46

conjugates, glucuronide and sulphate, were virtually identical.

However, the mean percentages of mercapturic acid and cysteine conjugates were higher in the alcoholic patients. This was not a reflection of an increased output of these metabolites in the 0 - 4 h period as during it the proportions of all the various conjugates were closer to those found with the healthy volunteers. The higher 0 - 24 h mean percentages were due to surprisingly high proportions of urinary mercapturic acid (8, 10, 10 & 14%) and cysteine (6, 7, 9, & 12%) conjugates in four alcoholic patients. There were no differences between the M.A.S.T. scores or plasma gamma glutamyl transferase concentrations in these four and the remainder of the alcoholic patients.

The mean 24 hour urine volume of the alcoholic patients (2151 ml) was slightly higher than those of the induced patients and healthy volunteers (1783 and 1798 ml respectively) due to an excessive fluid intake in one individual.

There were no significant differences between the three groups in the renal clearances of paracetamol and its glucuronide and sulphate conjugates (Table 3).

Individual differences in metabolism of either of the drugs did not appear to be related to smoking habits.

DISCUSSION

Induction of drug metabolising enzymes was confirmed in the patients taking anticonvulsants or rifampicin by the greatly shortened antipyrine half life and increased clearance without any change in the apparent volume of distribution. These patients had enhanced glucuronide conjugation of paracetamol compared to the healthy volunteers. They had a shorter plasma paracetamol half life and increased plasma glucuronide conjugate concentrations with an increased ratio of the AUC of the glucuronide to that of paracetamol. The increased glucuronide conjugation was reflected in corresponding differences in the proportions of paracetamol and its metabolites excreted with the induced patients excreting more glucuronide and less paracetamol or its sulphate conjugate than the healthy volunteers. Sulphotransferase does not appear to be induced in a similar fashion to glucuronyl transferase and sulphate conjugation was not increased in the induced patients.

There was no comparable evidence of drug metabolising enzyme induction in the alcoholic patients. Their antipyrine half lives and clearances were similar to those of the healthy volunteers and between the two groups there were no significant differences in paracetamol elimination and glucuronide or sulphate conjugation.

All our sixteen subjects claimed to have been drinking substantial amounts of alcoholic beverages for at least a few years and their classification as alcoholics was supported

supported by their M.A.S.T. scores. This test has been described as identifying with almost perfect accuracy individuals diagnosed as having alcoholism (Favazza and Pires, 1974). Only a third of our patients had raised plasma gamma glutamyl transferase concentrations. However, there is increasing evidence that this is not a reliable index of high alcohol intake alone.

Although alcohol is often listed as an inducer of hepatic microsomal enzymes, many of the studies on which this claim is based have considerable shortcomings (Sellers and Holloway, 1978). No correlation between antipyrine metabolism and alcohol consumption was found in 291 subjects from the Baltimore Longitudinal Study of Aging (Vestal et al, 1975). Although Vesell et al (1971) found that in six subjects modest alcohol consumption for three weeks was associated with a 4 - 37% reduction in antipyrine half life, alcohol does not appear to have an inducing action comparable to that of diphenylhydantoin or rifampicin.

There is evidence that acute alcohol administration inhibits hepatic drug metabolism (Rubin and Lieber, 1971; Sellers and Holloway, 1978). However, the small amounts of alcohol given in the present^{study}, at least eight hours after drug administration, were unlikely to have had significant effects. The same argument applies to the continuation of therapy in the induced patients and as with the alcohol, more closely mimics the situation found in clinical practice.

Previous studies in man on the effects of microsomal enzyme induction on paracetamol metabolism have given conflicting results. Perucca and Richens (1979) demonstrated reduced oral bioavailability of paracetamol which they attributed to increased first-pass metabolism in six epileptics compared with six normal subjects. The mean total body clearance and elimination rate were higher in the former group but the differences were not significant. No data was provided on the individual metabolites of paracetamol and the mercapturic acid and cysteine conjugates were not measured. However, Mitchell et al. (1974) found that pretreatment with 3 mg/Kg of phenobarbitone for five days had no effect on the plasma half life of paracetamol in seven healthy volunteers although formation of the mercapturic acid conjugate was increased.

The mercapturic acid and cysteine conjugates reflect the conversion of paracetamol to the potentially hepatotoxic metabolite. In animals the fraction of a dose converted to the mercapturic acid conjugate is highest in species most susceptible to the hepato toxicity of paracetamol and lowest in those most resistant. Similarly, inducing agents which potentiate paracetamol liver toxicity also increase mercapturic acid conjugate production while its formation is decreased by protective treatments (Jollow et al., 1974; Sato, Matsuda and Lieber, 1979).

In the present study no increase in the urinary excretion of mercapturic acid and cysteine conjugates was found in the induced patients. However, as these patients showed enhanced conversion of paracetamol to the glucuronide conjugate,

a corresponding fall in the output of the mercapturic acid and cysteine conjugates might have been expected similar to that occurring with the parent drug and its sulphate conjugate. Thus some induction of the oxidative metabolism of paracetamol cannot be excluded but does not appear to be of clinical significance at the dose used in this study.

Although there was no significant overall increase in mercapturic acid and cysteine conjugate production in the alcoholic patients, a number of individuals had relatively high outputs of these conjugates. There was no other evidence of microsomal enzyme induction and this may reflect selective activation of one of the multiple forms of cytochrome P-450 by alcohol. The present study leaves unresolved the question of whether therapeutic doses of paracetamol are potentially hepatotoxic in alcoholic patients and further work is planned.

Paracetamol metabolism is dose dependent (Prescott, 1980) Thus, although there is no evidence that anticonvulsant or antituberculous therapy may increase the risk of hepatotoxicity with therapeutic doses of paracetamol, this is not necessarily the case after overdosage.

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Part of this work is being published in the British Journal of Clinical Pharmacology (1981 - Volume 11) as a paper entitled "Effects of Microsomal Enzyme Induction on Paracetamol Metabolism in Man". A second paper based on the data from the alcoholic patients will also be submitted for publication.

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